

**Characterization of Resistance Mutation
Patterns Emerging Over Two Years During
First Line Antiretroviral Treatment with
Tenofovir DF or Stavudine in Combination
with Lamivudine and Efavirenz**

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XII International HIV Drug Resistance Workshop
Los Cabos, Mexico; 10-14 June 2003
Poster Presentation

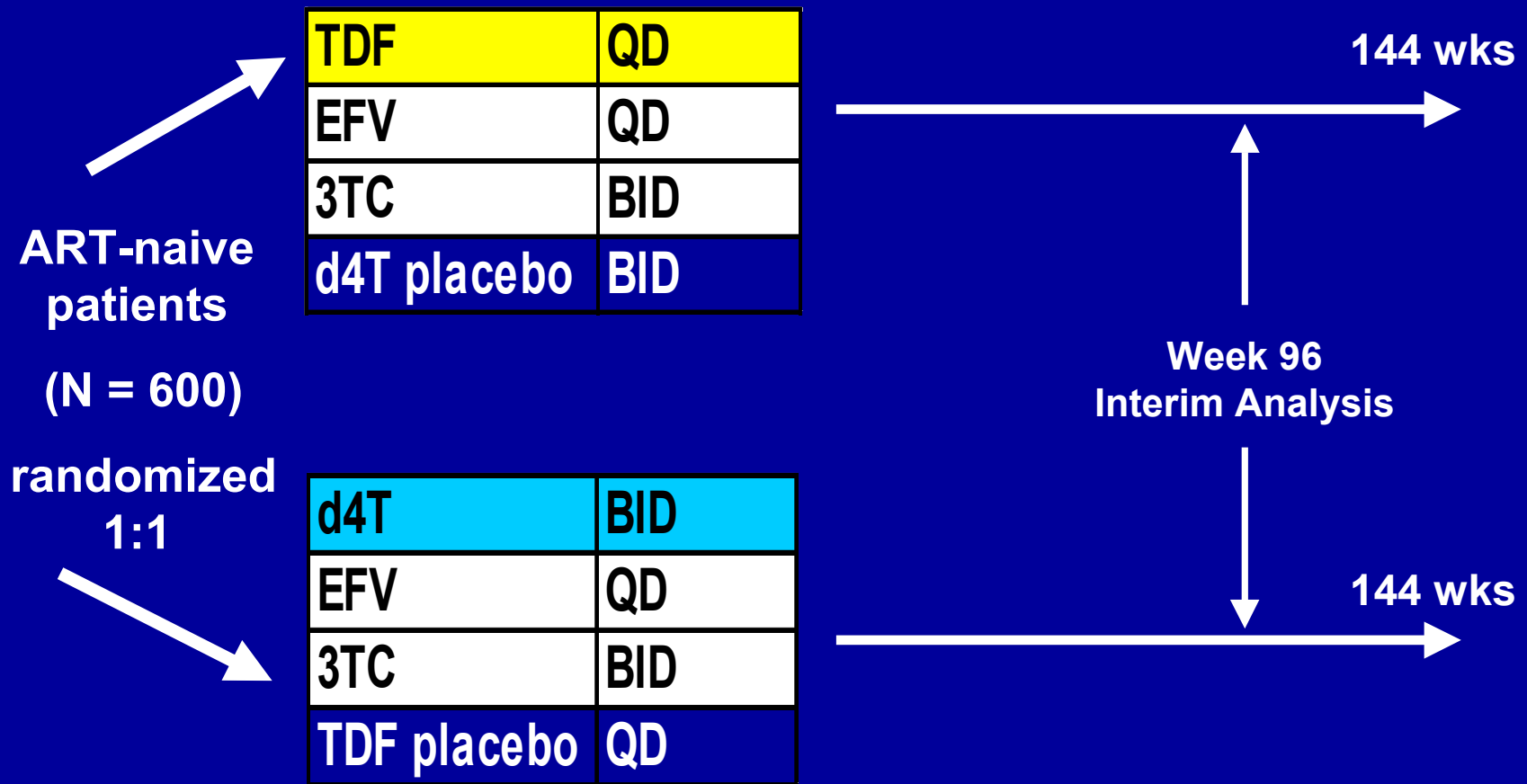
Introduction

- Tenofovir disoproxil fumarate (tenofovir DF, TDF) is a nucleotide analogue for the treatment of HIV-1 infection
- *In vitro*, tenofovir can select for the K65R mutation in HIV-1 RT which results in reduced susceptibility to tenofovir and some other NRTIs (see Results)
- The K65R mutation also results in reduced enzymatic efficiency (decreased K_{pol} and increased K_m) as well as reduced viral fitness (Selmi et al, 2001; White et al, 2002)
- Clinical studies in treatment-experienced patients have shown that the K65R mutation occurs in approximately 3% of patients after up to 96 weeks of tenofovir DF therapy
 - no increase in rate of development of K65R over time
 - development of K65R was not associated with HIV RNA rebound
 - no evidence for development of other types of resistance mutations to tenofovir DF
- The current study analyzes the development of resistance in treatment-naïve patients undergoing tenofovir DF therapy in combination with lamivudine (3TC) and efavirenz (EFV)

Methods

- Study 903 is a 144 week on-going Phase III clinical trial of tenofovir DF or stavudine (d4T) in combination with 3TC and EFV (Figure 1)
- In this interim analysis, plasma HIV was analyzed from all patients with virologic failure during the first 96 weeks (ITT analysis)
 - genotypic analyses performed by Virco (amino acids 1-400 of RT)
 - phenotypic analyses performed by ViroLogic (all licensed antiretroviral drugs)
- Virologic failure was defined as patients with > 400 copies/mL of HIV RNA at either:
 - week 48
 - week 96
 - early discontinuation
- Week 96 or last available sample was analyzed (last on-study)
 - median time from virologic failure to analysis was 17 weeks

Figure 1. Study 903 Design



- HIV RNA > 5000 copies/mL; no CD4 entry criteria

Table 1. Study 903 Baseline Characteristics

	TDF+3TC+EFV (n=299)	d4T+3TC+EFV (n=301)
Mean age (years)	36	36
Female	26%	25%
White	64%	64%
Mean HIV RNA (copies/mL)	81,300	81,300
Mean CD4 count (cells/mm ³)	276	282
HIV RNA >100,000 copies/mL	43%	42%
CD4 cell count < 200 cells/mm ³	39%	38%

Results: Table 2.
Week 96 Efficacy Summary

	TDF+3TC+EFV (n=299)	d4T+3TC+EFV (n=301)
• HIV RNA < 400 c/mL		
– Intent to Treat M=F	82%	78%
– As Treated	99%	96%
• HIV RNA < 50 c/mL		
– Intent to Treat M=F	78%	74%
– As Treated	95%	91%
• Mean CD4 cell change from baseline (cells/mm ³)	+261	+266

Table 3. Development of Resistance Mutations through Week 96 (ITT)

	Number of Patients (%)		p-value
	TDF+3TC+EFV (n=299)	d4T+3TC+EFV (n=301)	
Virologic failures	36 ² (12%)	38 (12.6%)	0.90
Any EFV-R ¹	22 ² (7.4%)	17 (5.6%)	0.41
EFV-R alone	8 ³ (2.7%)	6 (2.0%)	0.60
EFV-R + M184V/I	6 (2.0%)	9 (3.0%)	0.60
EFV-R + K65R	3 (1.0%)	1 (0.3%)	0.37 ⁴
EFV-R + M184V/I + K65R	5 (1.7%)	1 (0.3%)	0.12 ⁴
M184V/I alone	3 (1.0%)	3 (1.0%)	1.0
K65R alone	0	0	1.0
Wild-type or as baseline	11 (3.7%)	18 (6.0%)	0.25

¹ K103N, V106M, Y188C/L or G190A/S/E/Q (K103N in 27/39; others > 50 fold EFV-R with other mutations)

² Three patients (all in TDF arm) had > 4-fold EFV-R at baseline and developed additional EFV-R

³ One patient developed D67G+K70E+V75L and >20-fold 3TC resistance, but no tenofovir susceptibility change

⁴ Combined K65R comparison: p=0.06 (Fisher's Exact test)

Table 4. Non-K103N Patterns of NNRTI Resistance

NNRTI Resistance Mutations	Fold Change from Wild-Type		
	EFV	NVP	DLV
At Baseline			
V106I ¹ + V179D (n=1)	14.1	7.8	27.2
V108I + V179E (n=1)	3.8	7.7	4.6
Developed by week 96 ²			
V106M + V179D ³ (n=3)	>500	>50	>120
V179I + G190S (n=1)	>500	>50	5.1
G190Q (n=1)	>600	>65	>200
G190E (n=1)	>500	>44	30
V108I + Y181C + G190A (n=1, AG subtype)	>600	>96	>200
V179I + Y188L (n=1, NVP switch)	52	>72	9.8
V179G + Y181C + G190E (n=1)	>500	>43	>170

¹ Converted V106I to V106M during treatment and >900-fold EFV-R resulted

² No difference in development of non-K103N by treatment arm (32% and 29% for TDF and d4T, resp.)

³ V179D present at baseline

Table 5.
Phenotypic Susceptibility of NRTIs in
Presence of K65R (Virco Antivirogram, n=8)
 Fold Change Phenosense Assay (ViroLogic cut-off)

Patient	AZT (2.5)	d4T (1.7)	ddl (1.7)	ABC (4.5/6.5)	3TC (2.5)	TDF (1.4/4.0)
1 (+M184V)	0.3	0.9	3.7	6.2	>>	1.2
2 (+M184V)	0.5	1.0	3.0	7.0	>>	1.3
3 (+M184V)	0.3	0.8	1.9	4.6	>>	1.1
4	0.2	0.6	0.7	1.2	11	1.0
5	0.4	1.1	1.6	1.5	8.7	1.4
6 (+M184V)	0.9	0.8	1.2	1.3	>>	0.9
7	0.5	1.2	1.9	4.2	13.3	2.2
8	0.5	0.9	1.6	2.4	13	1.0
Mean	0.5	0.9	1.4/2.4*	2.3/4.8*	11/>>*	1.3

■ Fully Susceptible
 ■ Intermediate Susceptibility
 ■ Resistant

* M184V in addition

Table 6.

Replication Capacity and Other RT Mutations Developing in TDF Patients Developing K65R

Patient Number	Replication Capacity ¹	Tenofovir Susceptibility ²	Final RT Mutations Developing ³
1	NA	1.2	A62V, K65R, S68N, M184V
2	72%	1.3	K65R*, S68G/S, M184V
3	50%	1.1	K65R*, M184V
4	2%	1.0	K49R, K64R/K, K65R, G196R, K219R
5	16%	1.4	K65R, E194K
6	60%	0.9	K65R/K*, S68G/S, M184I, M230L
7	82%	2.2	A62V, K65R*, S68G, T139K, L228R
8	36%	1.0	K20R, K65R

¹ ViroLogic assay (% of wild-type control); ² Fold change from wild-type control

³ Excluding known NNRTI-resistance associated mutations

* K65R became undetectable during follow-up

- Mean viral replication capacity: 45% of wild-type

Table 7.
 Site-Directed Mutants of K65R with
 A62V or S68G

Fold Change PhenoSense Assay (Cut-Off)

Virus	TDF (1.4)	AZT (2.5)	d4T (1.7)	ddl (1.7)	ABC (4.5)	3TC (2.5)
K65R	1.7	0.4	1.6	1.6	2.3	9.7
K65R + S68G	1.9	0.6	1.8	1.6	2.4	9.0
K65R + A62V	1.8	0.5	1.4	1.3	1.6	6.3

■ Fully Susceptible

■ Intermediate Susceptibility

- Addition of A62V or S68G causing no significant changes in drug susceptibilities
- Combination under investigation

Table 8.
**Outcomes of TDF-Treated Patients
 with K65R (n=8)**

Patient Number	Next Regimen	Response (copies/mL)	Follow-up
1	TDF/AZT/LPV/r	<50	W120
2	TDF/3TC/ddI/LPV/r	<50	W108
3	ddI/d4T/IDV/r	<50	W120
4	ddI/IDV	<50	W120
5	AZT/3TC/SQV/r	<50	D/C W48
6	AZT/ddI/NFV	423	D/C W68
7	AZT/3TC/APV	1905	non-adherence M184V developed
8	AZT/3TC/LPV/r	NA	no additional follow-up yet

Table 9.
**Baseline Characteristics of TDF-Treated
 Patients with Virologic Failure**

	Median Baseline HIV RNA (copies/mL)	Median Baseline CD4 Cells (cells/mm ³)
Virologic Failure with		
K65R (n=8)	246,000*	24**
Other mutations (n=17)	121,000	126*
No mutations (n=11)	92,000	418
All TDF patients (n=299)	78,000	253

* p<0.05 versus all TDF patients

** p<0.01 versus all TDF patients

Characteristics of Treatment Response and Virologic Failure in Patients Developing K65R (n=8)

- All patients showed initial viral load decrease of $>2 \log_{10}$
 - 6/8 never achieved <50 copies before failure
 - no evidence of treatment non-adherence
 - median time to virologic failure 12 weeks (range 4-29)
- After failure and just before new regimen:
 - median $-0.9 \log_{10}$ reduction of HIV RNA from baseline
 - median 49 cell/mm^3 increase in CD4 from baseline
 - mean 45% replication capacity of wild-type

Conclusions

- EFV resistance (6.5%) and M184V (4.5%) were the most commonly observed mutations at failure
 - multiple non-K103N patterns of EFV-R observed
 - no significant differences between treatment arms
- K65R emerged less frequently
 - 8/299 patients (2.7%) in TDF arm; 2/301 (0.7%) in d4T arm
 - observed only with EFV resistance
 - associated with high HIV RNA and low CD4 at baseline
 - maintained median 0.9 log₁₀ HIV RNA decrease and 49 CD4 cell/mm³ increase from baseline
 - associated with decreased replication capacity and low-level phenotypic changes to tenofovir *in vitro*

Conclusions (continued)

- S68G and/or A62V occurred with K65R in 4 patients
 - no significant changes in drug susceptibilities
- No detectable tenofovir resistance in 78% (28/36) of TDF+3TC+EFV failure patients
 - no novel resistance patterns to TDF detected
 - no patients developed TAMs
- Effective second line regimens constructed in TDF-treated patients who developed K65R
 - 5/8 patients achieved and maintained <50 copies/mL
 - 2 patients maintained TDF therapy
 - median follow-up 76 weeks on new regimen
 - remaining patients without follow-up (n=2) or non-adherent (n=1)

Acknowledgements

- GSK and BMS for providing study drugs
- Staff at Virco and ViroLogic
- Investigators, study site personnel and patients in Study 903